1950

Physiological changes and freezing injury in maturing maize

Mohamed Mohamed Aboul-Ela
Iowa State College

NOTE TO USERS

PHYSIOLOGICAL CHANGES AND FREEZING INJURY IN MATURING MAIZE

by

Mohamed Mohamed Aboul-Ela

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1950

TABLE OF CONTENTS

INTRODUCTION

Injury by frost is a common cause of loss in farm crops, especially in northern latitudes. Corn or other grain may be damaged by an early frost in the fall. Frequently, not only is the yield reduced, but the entire crop is harder to handle, the keeping qualities are inferior, the palatability and feeding value are reduced, and the seed viability is lowered or destroyed.

The amount of injury has been found to be controlled by various factors. The main ones are temperature, duration of exposure, and moisture content of the material at the time of exposure. Rossman (56) and Kiesselbach and Ratcliff (32) found that the degree of injury in seed corn was increased when the moisture percentage was higher at the time of freezing. In maturing crops, the moisture content of the grain changes during development. It is clear, then, that a study of freezing injury in a crop would give better information if accompanied by a study of the physiological changes in the maturing crop at different stages of development.

The physical properties and chemical composition of corn and other grains have been studied by many investigators (3, 17, 20, 30, 31, 68. 69). In other research on the same crops the effect of freezing has been studied.

Very little, however, was done to relate the two lines of work.

This work was intended to study the effect of freezing on the physiological and chemical changes occurring in corn kernels harvested at different stages of maturity. Also it was planned to investigate the relationship between these changes and the freezing injury as shown in seed viability.

REVIEW OF LITERATURE

Physiological Changes in Maturing Kernels

The physical properties and the chemical changes occurring in the maturing kernels of corn, wheat, oats, barley, and other grains have been investigated extensively. As early as 1889, Shelton (64) reported on the changes of dry matter in corn, and the effect on yield when the corn was cut at different stages of maturity. The composition of maturing corn was studied by Hopper (30), Jones and Huston (31), Evans (20), and Lampe (33), and the composition of maturing wheat by Thatcher (68, 69), Eckerson (19), and Woodman and Engledow (73). To review such physiological changes, moisture and dry matter, carbohydrate distribution, and nitrogen distribution will be considered separately.

Moisture and dry matter

When we speak of maturity, we usually mean the ceasing of the chemical changes which end with the ripening of a plant. Aldrich (3) and Shaw and Loomis (63), working on maturity measurements in corn, define physiological maturity as the point at which the maximum dry weight of grain is attained, in contrast to the older estimations of maturity in terms of the moisture content of the grain.

In a corn kernel the amount of dry matter increases progressively with the advancement of growth. Just when the dry weight of the kernels reaches its maximum depends on various factors inside the plant and various others in the environment.

Shelton (64), reporting on four dent varieties, found that even when the husks were dry and the leaves beginning to turn, there was still a loss of from 10 to 12 per cent in the yield of shocked corn over that left standing. Robinson (55) reported that the maximum weight of 100 kernels of corn was attained when the moisture content dropped down to 40 per cent. On the other hand Aldrich (3) claims that the moisture in corn grain was 35 per cent when translocation ceased. He observed that there was an apparent development in the grain eight days after the immature plants were cut and shocked. He mentioned, however, that the narrow scope of his investigations did not justify final conclusions. Shaw (62) reports that early lines of corn gained dry weight until the moisture dropped below 30 per cent, but late lines were full weight at 40 per cent.

Dessureaux, Neal, and Brink (17) state that strains of corn reach 35 or 40 per cent moisture either before or after the increase in dry matter is completed. They conclude that the moisture content alone cannot be considered as an indication of maturity in specific cases, if we consider the grain to be mature at the time its maximum dry

weight is reached. Although the types of corn did not vary significantly in the percentage of moisture at the early stages of development, as found by Andrew, Brink, and Neal (4), differences in the rates of moisture decline for the sugary and non-sugary classes became apparent after the 20-day stage. The sweet types lost moisture more rapidly.

Carbohydrate distribution

Carbohydrates are not distributed uniformly in the different parts of the corn kernel. According to Hopkins, Smith, and East (29), the germ contains only 35 per cent of its dry weight as carbohydrates, while the horny gluten contains 70 per cent, and the other principal parts, horny starch and white starch, contain 90 to 95 per cent. Earle, Curtis, and Hubbard (18), analyzing the whole kernels of corn, found that the dry matter contained 1.7 per cent of sugars and 73.7 per cent of starch. When analyses of the endosperm and the germ were made separately, they found that the former contained 28 per cent of the total sugars in the kernel and 89 per cent of the starch, while the germ contained 70 per cent of the sugars.

Lampe's work (33) on the developing endosperm in maize shows that, at the successive stages, the reducing sugars were confined to the immature cells (cells that had no starch which were present uniformly in the early stage.

In older kernels the reducing sugars occurred in the immature cells at their bases. Sucrose was not abundant in the endosperm until 10 to 12 days after pollination. It increased rapidly and reached a maximum throughout the tissue approximately 15 days after pollination. During the entire period from pollination to complete maturity the total sugars in the endosperm continued to be abundant in the basal part of the tissue. The results of her microchemical studies agree with the results of the macroanalyses of Culpepper and Magoon (14) in showing that there is a gradual reduction in the amount of reducing sugars, and that there is a rise, followed by a decrease, in the amount of sucrose.

Lampe's data (33) also indicated that; (a) sucrose and not reducing sugars occurred in the embryo, (b) the amount of sucrose present in the endosperm was greater than that of reducing sugars, and (c) that the amount of total sugars present in the mature endosperm was greater in the cap than in the base. Matured kernels had no reducing sugars but had sucrose in the embryo.

Culpepper and Magoon (14) observed that in both sweet and field corn kernels the reducing sugars decreased gradually throughout the growing period. The sucrose was low at the beginning, then increased rapidly until 15 days after silking, and after that decreased slowly. Polysaccharides increased with age, but the rate of increase

varied in different varieties. The water extract of sweet corn kernels contained both soluble dextrins and soluble starch; that of field corn kernels contained soluble starch only.

On the basis of fresh weight of kernels at harvest Evans' data (20) on yellow field corn indicated that the percentages of starch and sucrose increased progressively, while that of the reducing sugars increased to a maximum at 22 days after silking and then dropped gradually. On the dry-matter basis, both sucrose and reducing sugar percentages dropped with maturation and remained constant after 43 days from silking, but starch rose steadily until 36 days, then reached a constant value.

According to the findings of Robinson (55), the percentage of reducing sugars in the dry matter of seed corn was highest when the kernels were in the dough stage (60 per cent moisture). The non-reducing sugar percentage decreased with advancing maturity. Generally the reducing sugars were low at all stages of growth when compared to non-reducing sugars.

Bernstein (9), however, found no significant decrease in the amount of reducing sugars of the endosperm of dent corn during development. Sucrose increased markedly during the first three weeks after pollination, after which it remained approximately constant. Immediately following

pollination, the reducing sugars were higher than the sucrose fraction. Between the first and the second week after pollination the sucrose increased until it exceeded the reducing sugars. Starch began to accumulate at a uniform rate two weeks after pollination.

The different types of corn studied by Andrew, Brink, and Neal (4) did not vary significantly in the quantities of reducing sugars which they contained, or in the rate of change of this carbohydrate during development. In the non-sugary types the water-soluble fraction of carbohydrates did not exceed 10 per cent, but it rose to 42 per cent in the sugary types. They mentioned that the sugary and waxy genes in corn primarily influenced the synthesis of polysaccharides in the maize endosperm cells. Secondarily affected were kernel weight, tenderness, and moisture content.

The most rapid increase in the starch of corn kernels was found by Wolf, MacMasters, Hubbard, and Rist (72) to occur between 12 and 20 days after pollination. At maturity, the starch granules had increased to more than three times their diameter at 13 days after pollination in both the waxy and dent types, and had increased to only twice their diameter in sweet corn. The dent corns had larger starch granules, followed by the waxy and the sweet corns. The water-binding capacity of the starches at 12 to 13 days

after pollination approximated 0.9 gram of water per gram of starch, and diminished to 0.3 gram per gram at 20 to 36 days.

Nitrogen distribution

Hopkins, Smith, and East (29) determined the nitrogen content of the different parts of field corn kernels. Calculating their results as crude protein, they found that the hulls were the poorest part, containing only about 4 per cent of their dry weight as protein. The next poorest in protein was the tip cap and white starchy parts, which contained 7 or 8 per cent. The horny starch, the germ, and the horny gluten parts contained 10 to 11, 19.6, and 24.6 per cent of their respective weights as protein. They calculated that, of the total protein contained in the kernel, 22, 40, and 20 per cent were in the horny gluten, the horny starch, and the germ parts, respectively. According to Earle, Curtis, and Hubbard (18) the endosperm of the corn kernel contains 75 per cent of the total nitrogen, with 22 per cent in the germ.

Spitzer, Carr, and Epple (67) report that the characteristic proteins of corn are zein, globulin, and glutelin. The first is the most abundant but there are three amino acids lacking in its formula, two of which, lysine and tryptophane, are essential to growth and to the development of

young animals. The others are complete proteins but are not present in sufficient amounts to produce maximum growth.

Hansen, Brimhall, and Sprague (22) found that zein constitutes 28 to 60 per cent of the total protein in corn kernels, and that zein is located in the endosperm and absent from the germ. They found also a high correlation between zein and total protein in dent corn. Csonka (13), however, noted that the proportion of zein to other proteins varied with the nitrogen content of the kernels.

Showalter and Carr (66) compared the different nitrogenous compounds in high- and low-protein corns. In the Champion White Pearl variety, the total nitrogen was found to be 2.95 per cent of the dry weight, and 56.64 per cent of the nitrogen was soluble in 90 per cent alcohol. In the Yellow Dent variety, the total nitrogen percentage was 1.29, of which 27.47 per cent was soluble in 90 per cent alcohol. In the high-nitrogen corn the amides and albumin were less, and the globulins formed a larger part of the total protein than in the low-nitrogen corn. It seemed to them that the increased proportion of zein (the part soluble in 90 per cent alcohol) was accompanied by a decrease in the quantity of glutelin.

Corn protein is thus shown to be of different types. Osborne and Clapp (51) extracted ground yellow dent corn with 85 per cent alcohol, and separated zein from this

extract by adding ice water. They extracted the residue with 0.2 per cent sodium hydroxide solution to obtain glutelin. This latter fraction is called zeanin by Larmour (34). Hydrolyzing zein with hydrochloric acid, they found it to be characterized, like other alcohol soluble proteins, by small percentages of arginine and histidine, no lysine, no tryptophane, and much ammonia and proline. Amino acids which were lacking in zein were all present in the alkali soluble protein.

Csonka (13) also found that zein of maize does not contain lysine or tryptophane, but the gluten contained 0.516 per cent tryptophane. In both yellow and white corn he noticed that the fraction of protein soluble in 1 per cent sodium chloride solution, that soluble in 80 per cent alcohol, and the insoluble make 16, 27, and 48 per cent of the total protein, respectively. He arranged the amino acids in corn protein according to decreasing amounts as follows: tyrosine, arginine, lysine, histidine, cystine, and tryptophane.

The total nitrogen as well as the proportions of the different proteins of corn are known to change during the development of the kernel. It was shown by Evans (20) that the total amount of nitrogen in the corn kernel increased progressively until maturity was reached. On the dry matter basis, however, the percentage of total nitrogen decreased (20, 74).

Zeleny's data (74) indicated that water-soluble, non-protein nitrogen, as a percentage of total nitrogen, decreased with maturity. The same was true with proteoses, but the prolamine nitrogen (zein) increased and the glutelin nitrogen showed a slight increase while the globulin remained nearly stable. The basic nitrogen of the water-soluble fraction increased as the corn approached maturity. He concluded that globulin and glutelin were synthesized at relatively constant rates throughout the growth period of the kernel, while zein was present in only small quantities in the early stages, and was synthesized at a very rapid rate as maturity approached. The rapid increase in zein was almost paralleled by the rapid decrease in water-soluble non-protein nitrogen, indicating that the water-soluble nitrogenous compounds were probably largely utilized in the synthesis of zein.

In wheat the total amount of nitrogen in a kernel increases steadily with maturation. At the same time, the percentage of protein nitrogen with respect to the total nitrogen, increases (49, 69, 73).

Freezing Injury

The injurious effect of cold temperatures on the viability of living organisms was noticed early in civilization. Since the ancient philosophers, many theories and

explanations of the cause of winter killing have been suggested. Comprehensive reviews on the subject have been written by Levitt (35) and by Luyet and Gehenio (42, 44, 45).

Cause of death

Luyet and Gibbs (46), and Chambers and Hale (12) observed a unicellular layer, such as onion scale epidermis, under a microscope. In slow freezing a considerable undercooling takes place before ice is formed. At the freezing point, congelation of the cell constituents starts at one end of the cell, and proceeds as a wave to the other end in approximately 1/2 to 3/4 of a second (46). If cooling was quick, the cells froze in sudden flashes one after the other (12, 46). At the moment of freezing the cells became opaque; then the opacity began to clear, indicating that numerous submicroscopic crystals are first formed in the cells, and then that these crystals increased considerably in size after their formation.

In slow cooling, ice crystals form first in the intercellular spaces and water diffuses to these crystals from the surrounding cells. If the temperature does not drop low enough to freeze the water within the cells, the protoplasm may be dehydrated. Whether the protoplasm will coagulate or not depends on the degree of dehydration,

salt content, and other factors.

Chambers and Hale (12) found a good illustration of the dehydrating effect of the freezing process in the epidermal cells of onion, which have a large central vacuole. As water diffused out to external ice crystals the protoplast shrank around the diminishing vacuole, and no internal freezing took place until the protoplast was destroyed. Retardation of crystallization in the interior was effected by the colloidal structure of the protoplasm, the higher solute concentrations following dehydration, and the existence of the plasma membrane. The latter effectively prevented the transmission of the crystallization process to the subcooled interior, even when ice was in contact with its external surface. Below a critical temperature, however, the subcooled interior was frozen and death ensued.

Living tissues exhibit a double freezing point. The first is the freezing point of the intercellular solvent water. The second is that of the cellular solutions.

If yeast cells (21), or moss leaves (43) are cooled rapidly to a very low temperature, the water vitrifies. In other words, ice crystals do not have time to form from the liquid phase. The liquid passes over to the vitrified state, and a glass is formed (21). In the ideal form the glass phase differs from the liquid phase chiefly by its high viscosity.

If the vitrified material is warmed rapidly, vitrofusion (44) occurs. That is, the glass goes to liquid directly and the protoplasm remains alive. On the other hand, if warming is slow, devitrification takes place, and crystals are formed first. The protoplasm is then killed. It appears, therefore, that the ice formation zone on the temperature scale is dangerous (44).

The idea that ice formation within the protoplasm is the real cause of death, is now held by many investigators. It then becomes important to investigate the conditions under which ice is formed. In this connection, one would first think of the moisture availability for ice formation. Barnes and Mathews (6) studied the x-ray diffraction of gelatin strips of various concentrations immersed in liquid air, then allowed to warm up either slowly or rapidly. Ice did not form in gels containing 50 to 63 per cent gelatin when cooled suddenly. Evidence of the presence of ice appeared after warming the gels or after cooling them slowly. A 70 per cent gel did not show ice no matter how treated.

In Luyet and Gehenio's experiment (43), moss leaves containing between 30 and 65 per cent water were immersed in liquid air, then warmed up either rapidly or slowly. The results showed that ice formation could be prevented, either because there was not enough water to freeze, as in

the leaves containing less than 30 per cent water, or because rapid freezing and warming prevented crystallization in the vitrified leaves with more than 30 per cent water.

Luyet and Condon (41) froze potato tissue and then counted the number of cells left alive. They found with slow freezing that the cells began to die when about 35 per cent of the water content was frozen. All of them were dead when more than 70 per cent of the water contained in the tissue was frozen.

The conclusion arrived at by many workers as a result of these experiments is that the cell remains alive whatever the temperature is, as long as no ice is formed inside it. As to why the water freezes in cells of some kinds or under certain conditions and not in cells of other kinds or under other conditions, Lipman (38) advanced the following hypothesis. He assumes that the cells that do not succumb to freezing have the water held in extremely fine spaces between the colloidal particles which make up the protoplasm. In these tiny spaces the forces of attraction between the colloid particles and water dominate the cohesive forces between the water molecules themselves. The cells that are easily injured have larger spaces between their colloidal particles and so the operating forces of attractions are dominated by the cohesive forces of the water molecules.

Becquerel (7) explains that when protoplasm dehydration by low temperatures occurs, chemical modifications in the colloidal particles and changes in configuration take place. The particles acquire the property of combining with each other to form a new coagulum of large micelles which never reform the original gel.

Effect on physical properties

When a green part of a plant is injured by cold, it changes color, wilts, and dies. Frozen corn kernels, however, after thawing seem to be soft with a spongy structure. Rossman (56) found that severely frozen corn seed, when dried, showed darkened color of the embryo, but some grains did not have a colored embryo and yet were low in germination. After further drying to 98°C much darker color showed in the frozen kernels.

Newton and McCalla (50) reported that the grade of wheat was lowered by frost, even in mature samples. McCalla and Newton (48) found that frost in wheat damaged the gluten quality, texture, and tenacity of flour. They concluded that the physical properties of gluten are more easily affected by frost than any chemical property studied in the grain or flour. When frozen at early stages of maturity, wheat kernels kept their green color (50, 71). The weight of 1,000 kernels was less in frozen samples, as was found

by Waldron (70) and Newton and McCalla (50). The frozen kernels are more subject to mold attack.

Effect on chemical composition

Not much work has been done on the chemical changes in corn as affected by freezing, which is the main object of this study. However, there was a hint in Schaible's paper (58) that immature corn caught by frost was lower in protein content as well as higher in moisture. When he calculated the protein to an equivalent moisture basis, however, it was about the same as in mature corn. Kiesselbach and Ratcliff (32) found no difference in the constituents of frozen and unfrozen corn grain.

Some work has been done on wheat and oats. Sharp (60) showed that freezing had little effect on the protein content of "mill stream," when wheat kernels were frozen at varying ages from 27 days to maturity. In the less matured samples there was a slight decrease in protein content associated with freezing. Using Marquis wheat, McCalla and Newton (48) found no change in the ash of frozen kernels treated at different stages of maturity. Kernels frosted at 28°F showed no apparent effect on percentages of total, salt-soluble, or non-protein nitrogen fractions. Exposures to 24, 22, and 18°F in the early stages reduced the percentage of total nitrogen and raised the percentage

of the other fractions. In flour from grain cut when containing less than 57 to 58 per cent dry matter, 28°F did not cause any change, but the lower temperatures led to an increasing percentage of reducing sugars. No differences in invert sugar could be attributed to the freezing effect.

The dry matter in wheat kernels was found to be reduced by severe freezing before maturity, diminishing the yield (39, 70).

Effect on viability

Grain seed harvested at an early age are capable of germination. Kernels of Hannchen barley, under Idaho conditions, normally mature 26 days after flowering. Harlan and Pope (23) cut the spikes at 6, 7, and 9 days after flowering and tested the seed for germination the next winter. The 6 days old seed germinated about 50 per cent, and almost all of the older lots germinated. Corn also germinated when harvested early (32, 56). When frozen, however, the viability of grain is weakened or lost. Kiesselbach and Ratcliff (32) in their study of the effect of freezing on corn ears at different stages of maturity, found that the loss of vitality depended upon temperature, duration of freezing, and moisture content of the seed. The same conclusions were arrived at by Rossman (56) in corn, and by Helgeson and Blanchard (27) in wheat.

When seed are dried, they are reported to stand extremely low temperatures. Adams (2) cooled moistened seed of pea, barley, flax, rutabagas, red clover, meadow fescue, and timothy in liquid air for 24 hours. All died except the timothy, which germinated 6 per cent. Timothy did not absorb water as fast as the other seeds from the moist soil where they were buried. All dried seed were viable after the treatment. He concluded that death occurred only if seed contained more than 12 per cent moisture.

When de Candolle (16) cooled seeds of Pisum sativum, Phaseolus vulgaris, and Foeniculum officinal for 4 days at less than -100°C, even though they were not predesiccated or cooled gradually, they remained alive. He also refrigerated different seeds at -41.93°C average temperature, 118 times on consecutive days. Approximately all the wheat, oats, and fennel survived the treatment, but only 13 of 60 Mimosa seed, and 10 of many Lobelia seed germinated. He believed that the grain were in a latent life and that their life ceased below a certain temperature. Then the protoplasm was completely inert and incapable of respiration or assimilation. In this case, they, in no way, suffered the severe low temperature. He believed the cell protoplasm of the seed which did not survive, was not completely inert.

More recently Lipman (37) found that desiccated seeds

of vetch, wheat, barley, Melilotus, tobacco, flax, buckwheat, spinach, and milo maize cooled at 1.35°K for over 2 hours and 4.2°K for over 40 hours, germinated after being warmed slowly. He concluded that living organisms in resting states, such as seeds and spores, did not need to respire in order to maintain the life pattern.

MATERIALS AND METHODS

Single cross WF9 x 38-11 field corn was used. Seed was planted on May 20 and May 23 in 1947 and 1948, respectively. Three stalks were left in a hill; hills were 40 inches apart. In each year, the field was one plot, 8 rows wide, extending from east to west. Each row contained about 80 hills. The whole plot was divided arbitrarily into two sampling areas (sub-plots) by a line in the middle passing north to south.

Sampling

The sampling and treatments were designed to be as follows: At different stages of maturity, ears from each area were snapped at random and divided randomly into 4 groups. One group of 10 ears was used immediately for moisture determinations of grain, husks, and cobs. A combined sample of kernels from the 10 ears was used as a source for 2, 40-gram samples killed in 80 per cent alcohol for chemical determinations. Two more 20-gram samples were used for moisture determinations. A second group of from 30 to 55 ears to be used as a control, was spread on wire racks built between corn rows in the field.

A third group of the same number of ears was put in the cold room immediately after harvest, exposed to a mild freezing, and then spread on the same racks. The object

was to obtain injury or partial killing of the kernels. The fourth group of the same number of ears was treated the same as the third group except for more severe freezing to obtain almost complete killing. Ears were taken directly from the field to the cold rooms, and after freezing they were kept at room temperature to warm before they were taken to the racks. An electric fan was operated in each cold room to maintain uniform temperature. The drying racks were built with 4 shelves. To give uniform drying conditions, the top shelf was covered with corn stalks.

Kernels for chemical analyses were killed in boiling 95 per cent ethanol. The quantity of alcohol was calculated so that, when combined with the moisture in the kernels, it made 80 per cent alcohol.

At intervals, from the corn left to dry on the racks, 6 to 10 ears from each group were taken to the laboratory for moisture and chemical sampling. This was repeated every 3 or more days.

In 1947 the first harvest was made on September 1. From each sub-plot, a group of ears was kept on racks as a control. A second group of ears was exposed to 20°F for 8 hours. A third group of ears was exposed to 18°F for 16 hours. Because of difficulty with the refrigerating system, these ears were kept overnight at 32°F and frozen the next day. The ears from each group were sampled for

moisture and weight determinations of kernels on individual ears, after 5, 13, and 37 days of drying in the field. Chemical samples were taken from the pooled kernels of each group of ears.

The second harvest was made September 7 and the same procedure was followed, except that ears were sampled 3, 10, and 33 days after harvest. The third harvest was made on September 13 and treatments were the same, but the ears were sampled 3, 10, and 31 days after harvest. The fourth harvest, on September 22, received more severe treatment, the mild freezing being 8 hours at 10°F and the severe one, 16 hours at 10°F. The ears were sampled 3, 10, and 25 days after harvest. A fifth harvest of 10 ears was made on October 7 for final chemical samples, moisture, and kernel weight determinations.

In 1948, the general procedure in sampling was about the same as in 1947, except that each time a combined sample of 10 ears was used for chemical samples, moisture, and kernel weight determinations. When moisture and weight of kernels only were desired, 5 ears were combined for sampling. No determinations were made of the weights or moistures of husks and cobs.

The first harvest in 1948 was made on August 26. The mild freezing was 8 hours at 20°F, and the severe was 8 hours at 0°F. For the ears exposed in the field, chemical

samples were taken 5 and 10 days after harvest. Moisture and weight of kernels was determined at 5, 10, 15, 20, and 30 days after harvest. The second harvest was made September 3. Treatments and intervals for sampling the drying ears were the same as in the first harvest, except that the 30-day sample was not taken.

The third harvest was made on September 11. Treatments were 8 hours at 20°F and 14 hours at 0°F for the mild and severe freezings, respectively. The fourth harvest was made on September 19. Exposures were 20 hours at 20°F and 30 hours at 0°F. Samples of ears were taken at 5, 10, 15, and 20 days for the third harvest, and at 5 and 10 days for the fourth one. A fifth lot of 10 ears was collected October 5 but no treatments were given.

Chemical Procedures

The samples killed in boiling 80 per cent alcohol were decanted 4 times, then extracted for 48 hours in Soxhlet extractors. For decantations and extractions, 80 per cent alcohol was used for the 1947 samples, and 70 per cent for the 1948 samples.

The grain was ground with a mortar and pestle before Soxhlet extraction. The extract of each sample was made to one liter volume and stored for later analyses. The residues were dried at 100°C, ground to 60 mesh, and stored.

In the alcohol extract, reducing sugars, sucrose, and alcohol soluble nitrogen were determined. For sugar analyses, the extract was cleared by the method described by Lind (36) p. 14. The procedure for reducing sugars and sucrose determinations in the cleared extract was the same as described by Hassid (25, 26). The method for nitrogen determination was as follows.

Ten ml. of alcohol extract was pipetted into a 100 ml. Kjeldahl flask; also ca 1.0 gram of K_2SO_4, ca 0.02 gram of copper selenite* ($CuSeO_3 \cdot 2H_2O$), 5 ml. of concentrated sulfuric acid, one drop of paraffin oil, and 2 glass beads were added. After digestion was completed, 25 ml. of distilled water was added to each flask while still warm.

After cooling the flasks, 20 ml. of 30 per cent NaOH solution was added carefully to the digest, and the ammonia in each flask was distilled immediately into 10 ml. of 4 per cent boric acid solution. The ammonia was then titrated against a standard HCl solution using a mixed indicator.**

---

*This catalyst was recommended for corn material by Dr. B. Brimhall of the Ag. Exp. Sta. Chem. Section at Iowa State College. The material was purchased from Hach Chemical and Oxygen Company, Ames, Iowa.

**The mixed indicator was 0.13 per cent methylene blue + 0.09 per cent methyl red in 95 per cent alcohol. It gives a purple color with acid and a green color with ammonia, the end point being a light gray.

The nitrogen was determined in the residue from the extract by the same method. About 0.05 to 0.08 grams of the dried samples were used. Amounts of reagents were the same as for the alcohol-soluble fraction.

Viability Tests

The dried kernels in the 1947 samples were tested for germination by Miss N. Bennett (8). She used two methods of germination on each ear in a study of tetrazolium chloride as a test reagent for freezing injury of corn seed. Of the two methods, only the results of germination in sand are reported here. No weights for seedlings were reported.

The first harvest samples were not tested by Miss Bennett. The seed from these samples saved for the germination test were badly damaged by insects and fungi because of poor storage conditions. The few sound seeds that were saved were germinated on blotting paper.

After the 1948 samples had been dried in corn driers, 10 kernels were taken at random from each of 10 ears from each treatment. The kernels were combined and germinated in sand in the green house. A randomized block design was used with 5 replicates for each treatment. After 12 days, seedlings were about 3 to 5 inches high. Combined weights for seedlings in each plot were recorded to obtain an average seedling weight. The top parts of seedlings, consisting of the epicotyl and leaves, were weighed.

On September 1, 1950, another experiment was made as follows. Eight ears of corn with an average moisture percentage of 56.3 were divided into two halves with their husks on. For each ear, one half was frozen 10 hours at $-10^{o}F$ and the other half was kept as a check. Out of the 8 check halves, 4 were tops and 4 were bottoms of the ear. After the treatment each half was sampled for moisture determination. The rest of each of the 16 halves with the husks removed, were kept in the laboratory under a fan for 20 hours. Then they were sampled for moisture again. Only 4 ears were sampled for chemical analyses. Sugars and nitrogen fractions were determined in each of the 8 halves separately.

RESULTS

Dry Weight per Kernel

The dry matter in 100 kernels increased with maturity. In the 1947 harvest, the dry matter per 100 kernels increased from 25 to 28.5 grams when the moisture percentage in the kernels dropped from 40 to 30, respectively. In the 1948 harvest, however, no such increase was observed. The amount of dry matter per 100 kernels did not change with drying in the field after harvest except in the early stages. A slight increase or decrease in the dry weight was probably due to sampling error.

The moisture percentages in both cobs and husks were higher than in the kernels at the early harvests. Moisture then declined rapidly in husks, and very slowly in the cobs in the later harvests compared to the kernels (table 1). The data for moisture losses and dry weight of 100 kernels are shown in tables 2 and 3. The frozen ears, without exception, dried at a slower rate than the unfrozen when both were spread unshucked on racks in the field. Graphs showing the rate of drying in the field are shown in fig. 1 and 2. In general, the rate of drying was uniform except in a few cases when drying was slowed by weather conditions.

The analysis of variance for the 1948 data is presented in table 4. The effect of freezing on slowing the

Table 1 The green weight and moisture percentage of husks and cobs of corn at different stages of maturity, 1947.

Moisture in kernels %	Husks		Cobs	
	Green wt. g.	Moisture %	Green wt. g.	Moisture %
64.9	137.0±10.17	76.3±0.98	193.1±8.55	73.3±1.09
58.3	88.8± 5.17	68.7±0.74	139.3±4.28	62.2±1.39
50.3	37.4± 3.90	61.0±2.31	108.5±5.89	67.9±1.03
39.5	22.2± 2.50	25.4±3.55	110.3±8.51	60.6±1.52
31.8	25.0± 2.55	18.7±1.92	110.8±4.74	60.6±0.70

Table 2 Effect of freezing on rate of drying of snapped ears in the field, and on dry weight of kernels, 1947.

Date of harvest	Moisture at harvest, %	Days drying	Moisture in kernels, %			Dry wt. 100 kernels, g.		
			Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
Sept. 1	64.9± 1.32	0	64.9±1.32	64.9±1.32	64.9±1.32	11.2±0.63	11.2±0.63	11.2±0.63
		5	52.3±1.34	51.0±0.81	60.2±1.42	12.0±0.77	13.0±0.66	11.3±0.83
		13	34.4±1.12	34.0±1.26	51.9±3.54	14.1±0.71	13.1±0.82	8.5±1.20
		37	19.1±0.65	20.2±0.63	---------	11.7±1.00	14.4±0.86	---------
Sept. 7	58.3± 1.27	0	58.3±1.27	58.3±1.27	58.3±1.27	15.2±0.82	15.2±0.82	15.2±0.82
		3	51.1±1.40	55.9±1.19	57.4±1.95	16.5±1.00	16.1±0.83	14.5±1.25
		10	37.9±0.98	55.0±1.72	51.6±2.22	17.7±0.69	13.1±0.87	13.5±0.97
		33	21.7±0.79	24.4±1.29	---------	17.8±0.79	14.7±0.70	---------
Sept. 13	50.3± 1.27	0	50.3±0.60	50.3±0.60	50.3±0.60	20.1±0.72	20.1±0.72	20.1±0.72
		3	45.3±0.76	50.2±1.61	46.2±1.17	21.4±0.82	19.5±1.36	18.6±1.00
		10	30.4±0.83	40.0±0.84	33.7±0.94	20.8±0.65	18.5±0.67	18.9±0.86
		31	20.5±0.67	23.5±1.58	17.6±0.68	20.5±0.85	19.5±0.74	19.0±0.70
Sept. 22	39.5± 0.72	0	39.5±0.72	39.5±0.72	39.5±0.72	25.0±0.74	25.0±0.74	25.0±0.74
		3	35.0±0.71	37.6±0.79	39.4±1.03	24.9±0.62	25.3±0.88	23.0±1.10
		10	28.8±0.90	28.8±1.59	29.9±1.35	25.1±1.11	22.7±0.88	23.5±0.62
		25	19.1±0.41	16.5±0.82	17.6±1.04	24.6±0.91	22.5±1.13	23.5±1.00
Oct. 7	31.8± 1.79	0	31.8±1.79	31.8±1.79	31.8±1.79	28.5±0.48	28.5±0.48	28.5±0.48

Table 3 Effect of freezing on rate of drying of snapped ears in the field and on the dry weight of kernels, 1948.

Date of harvest	Moisture at harvest, %	Days drying	Moisture in kernels, %			Dry wt. 100 kernels, g.		
			Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
Aug. 26	68.5	0	68.5	68.5	68.5	9.87	9.87	9.87
		5	54.4	65.9	66.6	12.70	11.25	9.80
		10	45.3	63.6	60.8	13.80	10.60	10.60
		15	37.7	56.6	53.4	14.45	11.35	10.35
		20	34.6	53.7	39.1	16.05	9.30	9.35
		30	26.2	34.2	27.4	14.50	8.95	7.65
Sept. 3	56.9	0	56.9	56.9	56.9	17.10	17.10	17.10
		5	45.7	56.8	48.7	18.20	16.75	16.90
		10	39.1	51.4	48.5	19.70	17.25	17.45
		15	27.1	40.9	35.7	19.75	16.53	16.30
		20	27.9	38.6	35.3	18.55	17.00	16.65
		25	26.6	33.0	27.4	18.70	14.85	16.55
Sept. 11	46.6	0	46.6	46.6	46.6	23.30	23.30	23.30
		5	37.9	44.1	41.9	23.20	23.25	23.45
		10	30.0	35.5	32.0	23.10	22.95	23.65
		15	30.8	36.9	31.6	23.60	22.45	22.70
		20	26.0	30.9	26.9	23.45	22.60	20.35
Sept. 18	37.8	0	37.8	37.8	37.8	28.70	28.70	28.70
		5	34.0	37.2	38.0	27.65	28.50	27.40
		10	29.0	31.8	31.7	28.10	28.20	26.50
Oct. 5	24.8	0	24.8	24.8	24.8	28.95	28.95	28.95

Table 4 Analysis of variance for moisture percentages, 1948.

Source of error	Degrees of freedom	Sum of squares	Mean square
Reps (R)	1	52.07	52.07
Stage of harvest (H)	3	5988.37	1996.12**
Error A (R x H)	3	18.46	6.15
Treatments	6	1549.21	
Freez. vs control	1	637.06	637.06**
Mild vs severe freez.	1	41.18	41.18**
Drying effect	1	425.70	425.70**
Drying 5 vs 10 days	1	438.02	438.02**
Remainder	2	7.25	
Treatment x H	18	363.37	20.19**
Error B	24	53.93	2.25
Total	55	8025.41	

**Significant at the 1 per cent level.

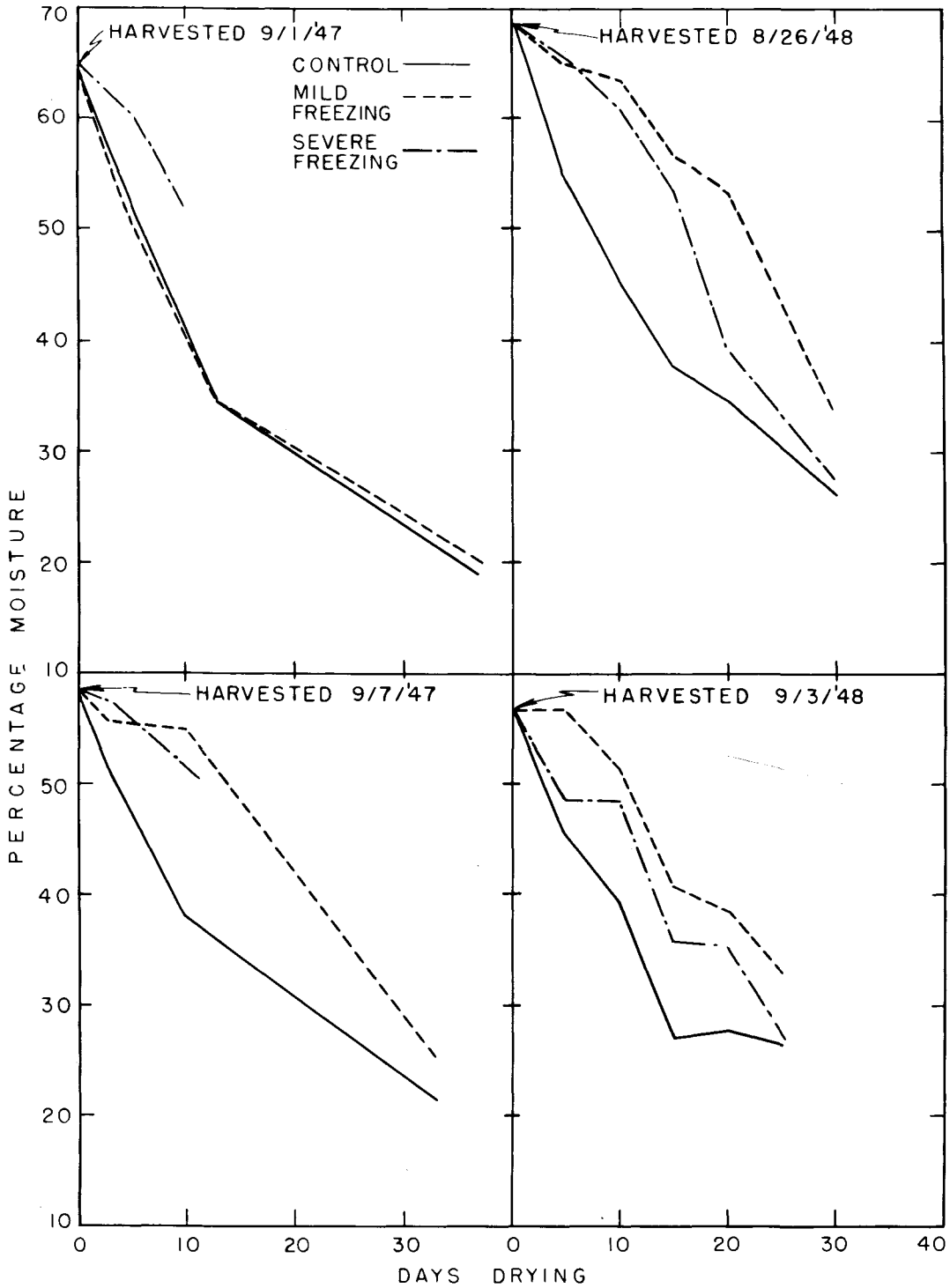

Fig. 1 Rate of drying of corn kernels as affected by freezing.

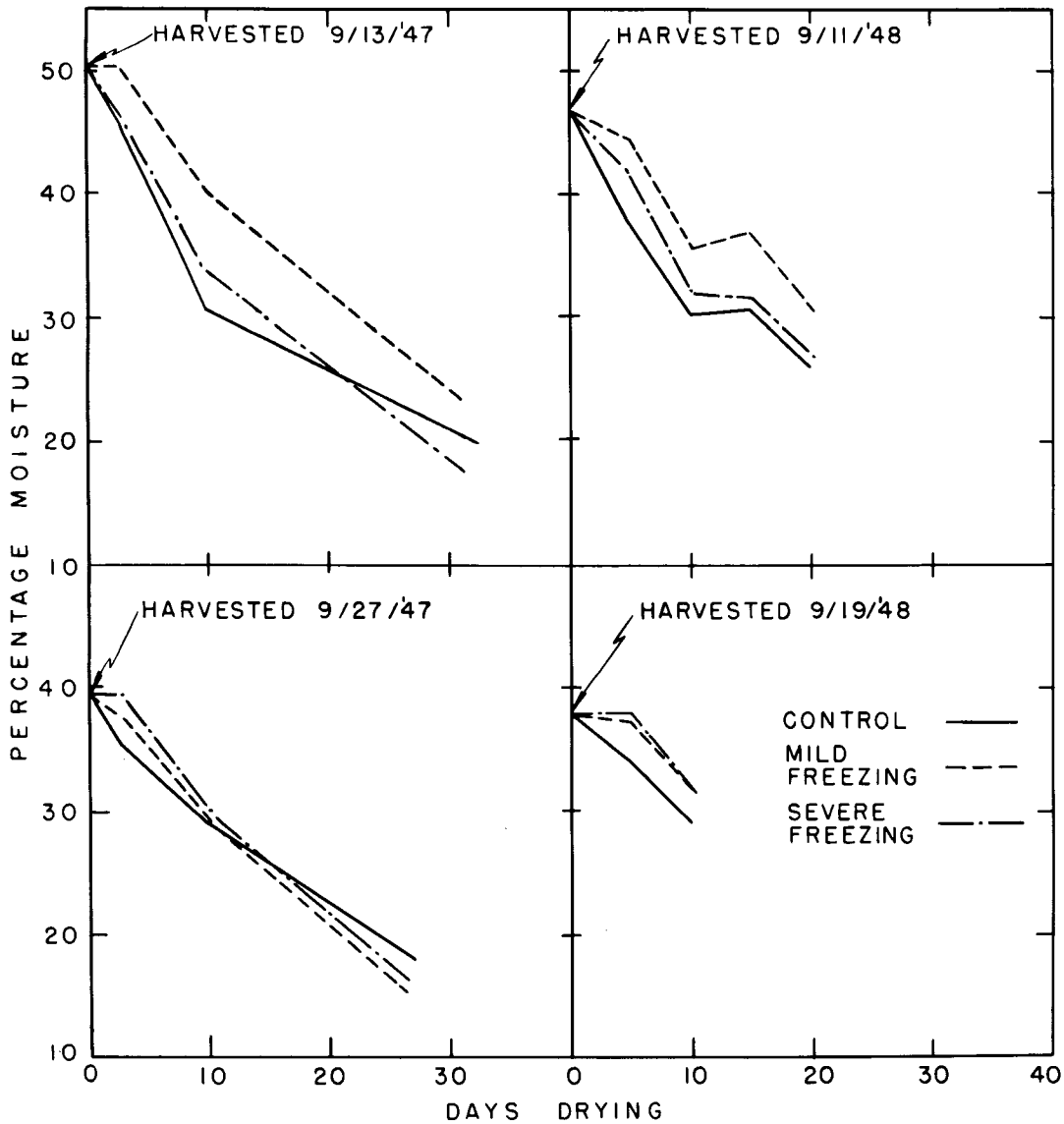

Fig. 2 Rate of drying of corn kernels as affected by freezing.

rate of drying was highly significant. Also, the mildly frozen ears showed a significantly slower rate of drying than the severely frozen. In the 1950 samples, however, it was found that the kernels from the frozen and shucked ear-halves lost moisture slightly faster than the unfrozen halves. In a 20-hour period under the fan the frozen halves of the ears lost an average of 21.5 per cent of moisture while the unfrozen halves of the same ears lost an average of 19.1 per cent. In table 5 it can be observed that in 7 out of 8 cases the frozen lost moisture faster than controls. The varying responses may have been due to the presence or absence of shucks and the effect of freezing on the opening of the shucks.

Seed Viability

The germination percentage was greatly reduced by freezing. In tables 6 and 7 it is clear that severe freezing caused more damage to seed than mild freezing. In interpreting the data it must be remembered that freezing treatments were intensified for the later harvests.

When the green weights of epicotyls were compared (table 8), freezing treatments gave a lighter average epicotyl weight, and severe freezing gave the lightest weight for epicotyls considering each stage of harvest alone. The weight of epicotyls within each treatment tended to

Table 5 Effect of freezing on the rate of drying of half ears without husks, 1960.

Ear	Percentage of moisture lost in 20 hours under fan		
	Control	Frozen	Difference
1	18.27	20.15	1.88
2	13.49	15.73	2.24
3	21.29	22.62	1.33
4	19.45	25.50	6.05
5	20.22	22.95	2.73
6	25.82	24.96	-.86
7	18.85	21.06	2.21
8	15.36	19.42	4.06
Total	152.75	172.39	19.64
Mean	19.1	21.55	2.45

Table 6 Effect of freezing on germination, 1947.

Moisture at harvest %	Germination percentage		
	Control	Mild freez.	Severe freez.
64.9	64.5±1.61	38.0±1.63	----------
58.3	97.0±0.82	76.4±3.04	13.0±5.39
50.3	98.9±0.53	52.6±7.54	18.4±3.65
39.5	98.1±0.78	12.3±5.40	0.5±0.34

Table 7 Effect of freezing on germination, 1948.

Moisture at harvest %	Germination percentage		
	Control	Mild freez.	Severe freez.
68.5	99	32	5.5
56.9	98	76	41.0
46.6	98	81	2.5
37.8	100	75	7.0

Table 8 Effect of freezing on green weight of epicotyls, 1948.

Moisture at harvest %	Average fresh weight of one epicotyl g.		
	Control	Mild freez.	Severe freez.
68.5	0.504	0.465	0.373
56.9	0.660	0.591	0.549
46.6	0.784	0.648	0.440
37.8	0.884	0.656	0.603

increase with the maturity of seed at harvest, but this trend is obscured by the irregularly more severe freezing treatments employed on the more mature samples (table 7).

Sugars

Data for the percentage of reducing sugars and sucrose with respect to the dry matter are summarized in tables 9 and 10. Analyses of variance for the 1948 data for reducing sugars are presented in table 11, and for sucrose in table 12. Generally, the percentage of sugars decreased with maturity.

In the 1947 crop, the reducing sugar percentage was 1.61 in the first harvest (65 per cent moisture), then dropped to 1.15, 0.87, 0.62, and 0.48 for the second, third, fourth, and fifth harvests, respectively. The sucrose percentage was much higher at each stage. For the first harvest, the percentage of sucrose was 3.98 and it decreased gradually to 1.32 for the fifth harvest. In the 1948 crop the same trend occurred (table 10).

When the ears of each harvest were dried in the field, the percentages of both reducing sugars and sucrose decreased gradually. The rate of decrease of reducing sugars, however, was significantly slowed down by freezing, as shown in fig. 3 and table 11. This result was similar to that obtained by McCalla and Newton (48) in wheat. Both the

Table 9 The changes in sugars occurring in maturing corn kernels as affected by drying and freezing, 1947.

Moisture at harvest, %	Days drying	Red. sug., % dry wt.			Sucrose, % dry wt.		
		Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
64.9	0	1.61	1.61	1.61	3.98	3.98	3.98
	5	1.07	1.13	1.96	2.58	1.50	0.96
	13	0.52	0.59	1.78	1.31	1.38	0.00
	37	0.40	0.35	----	1.30	1.40	----
58.3	0	1.15	1.15	1.15	4.13	4.13	4.13
	3	0.99	1.04	1.30	1.48	1.48	1.08
	10	0.49	0.83	0.57	0.95	0.67	0.20
	33	0.31	0.35	----	1.46	1.58	----
50.3	0	0.87	0.87	0.87	2.88	2.88	2.88
	3	0.85	0.85	1.17	0.84	1.50	1.49
	10	0.44	0.55	0.54	0.90	0.60	0.39
	31	0.47	0.46	0.39	1.14	1.26	0.22
39.5	0	0.62	0.62	0.62	2.56	2.56	2.56
	3	0.91	1.42	0.94	1.47	1.74	1.50
	10	0.50	0.72	1.34	1.26	1.62	0.97
	25	0.54	0.78	0.62	1.32	0.77	0.61
31.8	0	0.48	0.48	0.48	1.32	1.32	1.32

Table 10 The changes in sugars in maturing corn kernels as affected by drying and freezing, 1948.

Moisture at harvest, %	Days drying	Red. sug., % dry wt.			Sucrose, % dry wt.		
		Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
68.5	0	2.63	2.63	2.63	5.33	5.33	5.33
	5	1.00	1.52	1.74	1.24	1.03	0.84
	10	0.76	1.28	1.09	1.34	1.02	0.57
56.9	0	1.19	1.19	1.19	3.23	3.23	3.23
	5	0.87	0.87	0.84	1.08	0.73	1.51
	10	0.61	0.65	0.58	1.01	0.85	0.50
46.6	0	0.86	0.86	0.86	2.83	2.83	2.83
	5	0.64	0.69	0.87	1.08	0.78	0.30
	10	0.43	0.50	0.58	0.98	0.95	0.28
37.8	0	0.70	0.70	0.70	2.13	2.13	2.13
	5	0.48	0.87	0.71	1.01	1.50	0.60
	10	0.62	0.78	0.70	1.03	1.11	0.59
24.8	0	0.50	0.50	0.50	1.24	1.24	1.24

mild freezing and severe freezing had about the same effect on the rate of decrease in reducing sugars (table 11).

On the other hand, freezing accelerated the rate of decrease in sucrose significantly (table 12), and severe freezing had more effect than mild (fig. 4). McCalla and Newton (48) found no effect of freezing on sucrose of wheat.

In tables 11 and 12 the interactions (treatment x H) are highly significant, indicating that the treatment effect was different from one harvest to the other.

Data for 1950 (table 13) show that the percentage of reducing sugars in the frozen sample after drying was twice what it was in the controls. The percentage of sucrose was the same.

The quantities of reducing sugars and sucrose in 100 kernels were calculated from the dry weights and the respective percentages of the sugars. Data for 1948 are recorded in table 14. The reducing sugars per 100 kernels decreased slowly with maturity while the sucrose remained nearly constant until 40 per cent moisture was reached, then declined. The sequence is in agreement with previous investigations (9, 55). The amounts of sugars decreased with drying. Freezing slowed the rate of decrease in reducing sugars but accelerated it in sucrose (table 14).

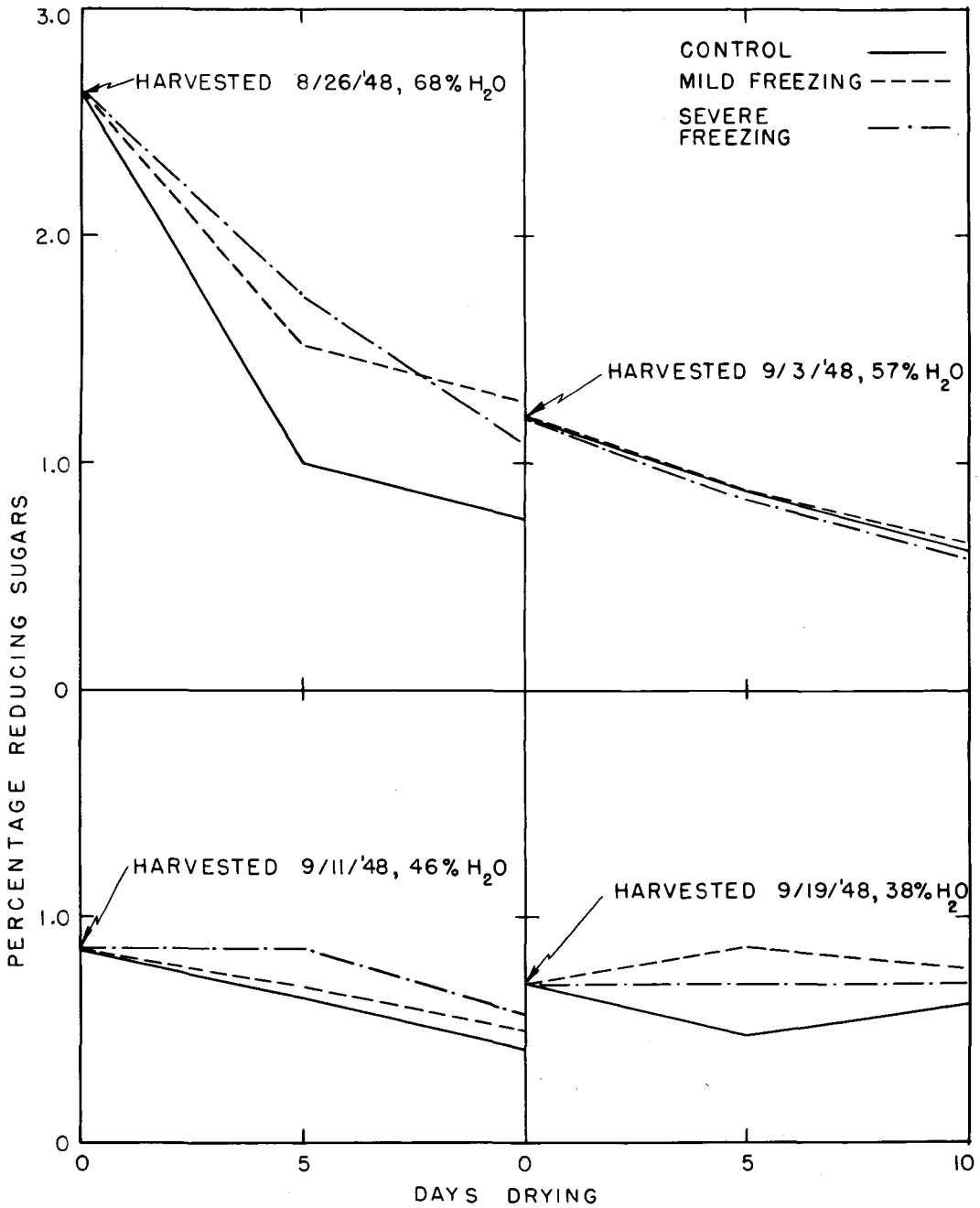

Fig. 3 Effect of freezing on the percentage of reducing sugars in the dry matter of kernels after field drying, 1948.

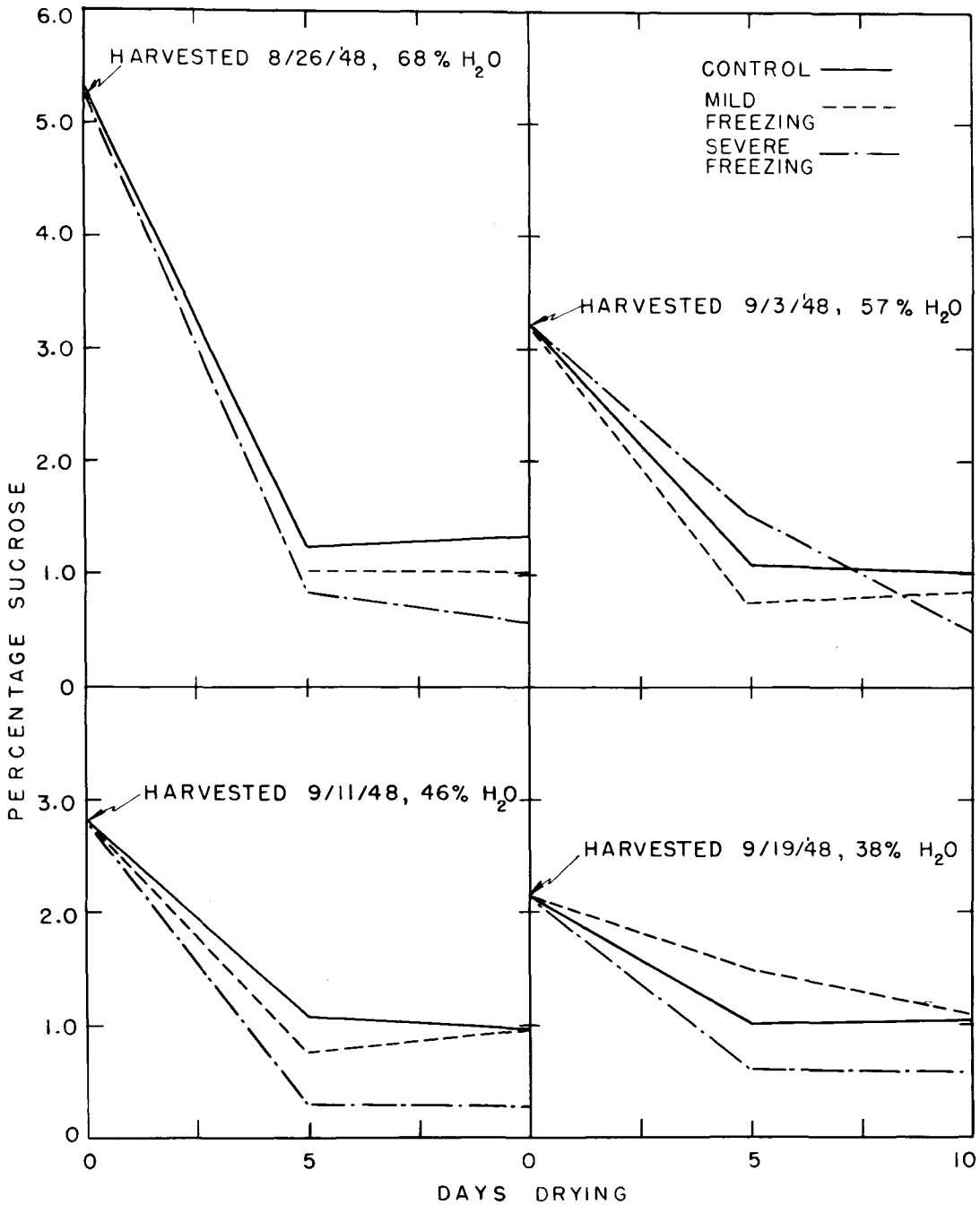

Fig. 4 Effect of freezing on the percentage of sucrose in the dry matter of kernels after field drying, 1948.

Table 11 Analysis of variance of reducing sugar data, 1948.

Source of error	Degrees of freedom	Sum of squares	Mean square
Reps (R)	1	0.0302	0.0302
Stage of harvest (H)	3	5.2962	1.7654**
Error A (R x H)	3	0.0543	0.0181
Treatments	6	3.2342	
Freez. vs control	1	0.6017	0.6017**
Mild vs severe freez.	1	0.0098	0.0098
Drying effect	1	1.8040	1.8040**
Drying 5 vs 10 days	1	0.6816	0.6816**
Remainder	2	0.1371	
Treatment x H	18	2.6959	0.1498**
Error B	24	0.2576	0.0107
Total	55	11.5684	

**Significant at the 1 per cent level.

Table 12 Analysis of variance of sucrose data, 1948.

Source of error	Degrees of freedom	Sum of squares	Mean square
Reps (R)	1	0.0005	0.0005
Stage of harvest (H)	3	2.6684	0.8895**
Error A (R x H)	3	0.0903	0.0301
Treatments	6	42.9573	
Freez. vs control	1	0.6852	0.6852**
Mild vs severe freez.	1	0.7595	0.7595**
Drying effect	1	41.2300	41.2300**
Drying 5 vs 10 days	1	0.1261	0.1261*
Remainder	2	0.1565	
Treatment x H	18	11.7357	0.6520**
Error B	24	0.5654	0.0236
Total	55	58.0176	

*Significant at the 5 per cent level.
**Significant at the 1 per cent level.

Table 13 Effect of freezing on changes in sugars in ears dried without husks, 1950.

Ear	Reducing sugar, % dry weight		Sucrose, % dry weight	
	Control	Frozen	Control	Frozen
1	0.918	1.958	1.195	1.148
2	0.786	1.720	0.943	0.793
3	1.022	1.926	1.022	1.203
4	0.977	1.831	1.096	1.091
Ave.	0.926	1.859	1.064	1.059

Table 14 Changes in the quantities of sugars in maturing corn kernels as affected by drying and freezing, 1948.

Moisture at harvest, %	Days drying	Mg. red. sug. in 100 kernels			Mg. sucrose in 100 kernels		
		Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
68.5	0	259	259	259	526	526	526
	5	127	171	170	158	116	82
	10	105	135	115	184	108	59
56.9	0	204	204	204	552	552	552
	5	153	146	141	195	122	254
	10	120	112	101	198	146	86
46.6	0	200	200	200	658	658	658
	5	149	159	204	249	182	71
	10	98	114	137	225	217	66
37.8	0	201	201	201	588	588	588
	5	133	248	286	278	427	251
	10	173	220	184	302	313	155
24.8	0	143	143	143	357	357	357

Nitrogen

The data of 1947 (table 15) and of 1948 (table 16) show that the percentage of total nitrogen in the dry matter of maturing kernels decreased steadily and slowly until development was completed. This result is in agreement with others (20, 31, 55, 74). The percentage of alcohol insoluble nitrogen remained constant while that of the alcohol soluble decreased gradually. Zeleny (74) reported that on the basis of total nitrogen the percentage of globulins and glutelins remained constant while that of zein increased with maturity. In the 1947 harvests, the percentage of alcohol-soluble nitrogen decreased from approximately 0.63 in the first harvest to 0.48 in the fifth harvest. In the 1948 harvests, however, the percentage dropped from approximately 0.93 to 0.19. The difference in the rate of decrease may be because 80 per cent alcohol was used for extraction of the 1947 samples and 70 per cent alcohol was used for the 1948 samples, or because of seasonal effects, or both.

The percentage of alcohol-soluble nitrogen decreased when the ears were dried in the field after harvest, while that of insoluble and total nitrogen increased (tables 15 and 16, and fig. 5). Analysis of variance for the 1948 data (tables 17, 18, and 19) indicates that the increase in total and insoluble nitrogen with drying were both

Table 15 Changes in nitrogen fractions of maturing corn kernels as affected by drying and freezing, 1947.

Moisture at harvest, %	Days drying	Nitrogen, % dry weight								
		Alcohol soluble			Insoluble			Total		
		Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
64.9	0	0.631	0.631	0.631	1.210	1.210	1.210	1.841	1.841	1.841
	5	0.636	0.839	0.959	1.290	1.120	0.980	1.926	1.959	1.939
	13	0.772	0.855	0.989	1.210	1.270	1.020	1.982	2.125	1.959
	37	0.988	0.767	-----	1.190	1.150	-----	2.178	1.917	-----
58.3	0	0.754	0.754	0.754	0.990	0.990	0.990	1.744	1.744	1.744
	3	0.803	0.771	0.928	1.060	1.010	1.050	1.863	1.781	1.978
	10	0.767	0.667	0.708	1.160	1.110	1.060	1.927	1.777	1.768
	33	0.699	0.717	-----	1.100	1.180	-----	1.799	1.897	-----
50.3	0	0.708	0.708	0.708	1.000	1.000	1.000	1.708	1.708	1.708
	3	0.643	0.707	0.708	1.120	1.040	1.010	1.763	1.747	1.718
	10	0.657	0.636	0.729	1.050	1.030	1.010	1.707	1.666	1.739
	31	0.898	0.634	0.633	1.040	1.080	1.060	1.938	1.714	1.693
39.5	0	0.699	0.699	0.699	1.000	1.000	1.000	1.699	1.699	1.699
	3	0.703	0.735	0.744	1.040	1.060	1.050	1.743	1.795	1.794
	10	0.559	0.579	0.650	1.040	1.020	1.050	1.599	1.599	1.700
	25	0.626	0.665	0.651	1.020	1.080	0.920	1.646	1.745	1.561
31.8	0	0.481	0.481	0.481	1.080	1.080	1.080	1.561	1.561	1.561

Table 16 Changes in nitrogen fractions of maturing corn kernels as affected by drying and freezing, 1948.

Moisture at harvest, %	Days drying	Nitrogen, % dry weight								
		Alcohol soluble			Insoluble			Total		
		Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
68.5	0	0.931	0.931	0.931	1.453	1.453	1.453	2.384	2.384	2.384
	5	0.934	0.826	0.964	1.804	1.590	1.422	2.738	2.416	2.386
	10	0.733	0.836	0.943	1.769	1.640	1.575	2.502	2.476	2.518
56.9	0	0.713	0.713	0.713	1.452	1.452	1.452	2.165	2.165	2.165
	5	0.491	0.706	0.656	1.634	1.535	1.474	2.125	2.241	2.130
	10	0.457	0.392	0.551	1.942	1.835	1.689	2.399	2.227	2.240
46.6	0	0.483	0.483	0.483	1.412	1.412	1.412	1.895	1.895	1.895
	5	0.343	0.306	0.562	1.585	1.594	1.593	1.928	1.900	2.155
	10	0.380	0.416	0.514	1.424	1.439	1.477	1.804	1.855	1.991
37.8	0	0.329	0.329	0.329	1.450	1.450	1.450	1.779	1.779	1.779
	5	0.195	0.422	0.429	1.662	1.517	1.436	1.857	1.939	1.865
	10	0.239	0.380	0.481	1.659	1.674	1.456	1.898	2.054	1.937
24.8	0	0.187	0.187	0.187	1.393	1.393	1.393	1.580	1.580	1.580

Table 17 Analysis of variance for alcohol-soluble nitrogen data, 1948.

Source of error	Degrees of freedom	Sum of squares	Mean square
Reps (R)	1	0.009912	0.009912
Stage of harvest (H)	3	2.486172	0.828724**
Error A (R x H)	3	0.014460	0.004820
Treatments	6	0.294785	
Freez. vs control	1	0.168170	0.168170**
Mild vs severe freez.	1	0.053301	0.053301**
Drying effect	1	0.023954	0.023954
Drying 5 vs 10 days	1	0.038138	0.038138*
Remainder	2	0.011222	
Treatment x H	18	0.234058	0.013003
Error B	24	0.162623	0.006776
Total	55	3.202010	

*Significant at the 5 per cent level.
**Significant at the 1 per cent level.

Table 18 Analysis of variance for insoluble nitrogen data, 1948.

Source of error	Degrees of freedom	Sum of squares	Mean square
Reps (R)	1	0.042713	0.042713
Stage of harvest (H)	3	0.177641	0.059214
Error A (R x H)	3	0.021459	0.007153
Treatments	6	0.456383	
Freez. vs control	1	0.168063	0.168063**
Mild vs severe freez.	1	0.062560	0.062560**
Drying effect	1	0.174045	0.174045**
Drying 5 vs 10 days	1	0.044095	0.044095*
Remainder	2	0.007620	
Treatment x H	18	0.432926	0.024051**
Error B	24	0.144415	0.006017
Total	55	1.275538	

*Significant at the 5 per cent level.
**Significant at the 1 per cent level.

highly significant though the decrease in the alcohol-soluble nitrogen was not.

A comparison between the effect of 5 days and 10 days drying indicates (tables 17, 18, and 19) that; (a) there was a change in both alcohol-soluble and insoluble nitrogen during this period, and (b) there was no change in the total nitrogen. Most of the change in total nitrogen occurred in the first 5 days. The significant interaction (treatment x H) in tables 18 and 19 indicates that the effect of freezing was not the same for each harvest.

Freezing as indicated in tables 15 and 16 and fig. 6 showed both the decrease of alcohol-soluble nitrogen and the increase of insoluble and total nitrogen with drying.

In a comparison between the effect of mild and severe freezing on the nitrogen fractions (table 17, 18, and 19), a significant difference was found for all fractions except total nitrogen.

Data of 1950 (table 20) show that the percentage of alcohol-soluble nitrogen after drying in the laboratory was high in the frozen compared to the control half ears. The percentage of the insoluble nitrogen, however, was lower in the frozen halves. Both results are in harmony with 1947 and 1948 data.

The quantities of nitrogen per 100 kernels for 1948 data were calculated and are recorded in table 21. It is

Table 19 Analysis of variance for total nitrogen data, 1948.

Source of error	Degrees of freedom	Sum of squares	Mean square
Reps (R)	1	0.093486	0.093486*
Stage of harvest (H)	3	3.402105	1.134035**
Error A (R x H)	3	0.024844	0.008281
Treatments	6	0.076070	
Freez. vs control	1	0.000000	0.000000
Mild vs severe freez.	1	0.000385	0.000385
Drying effect	1	0.068943	0.068943**
Drying 5 vs 10 days	1	0.000237	0.000237
Remainder	2	0.006505	0.006505
Treatment x H	18	0.451087	0.025060**
Error B	24	0.167951	0.006998
Total	55	4.215543	

*Significant at the 5 per cent level.
**Significant at the 1 per cent level.

Table 20 Effect of freezing on the nitrogen fractions after drying, 1950.

Ear	Alcohol soluble N., % dry weight			Insoluble N., % dry weight		
	Control	Frozen	Increase	Control	Frozen	Decrease
1	0.594	0.665	0.070	0.897	0.799	0.098
2	0.550	0.603	0.053	0.788	0.692	0.096
3	0.556	0.651	0.095	0.880	0.835	0.045
4	0.671	0.718	0.047	0.896	0.833	0.066
Ave.	0.593	0.659	0.066	0.865	0.790	0.075

Table 21 Changes in the nitrogen fractions of maturing corn kernels as affected by drying and freezing, 1948.

Moisture at harvest, %	Days drying	Mg. nitrogen in 100 kernels								
		Alcohol soluble			Insoluble			Total		
		Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.	Control	Mild freez.	Severe freez.
68.5	0	92	92	92	144	144	144	236	236	236
	5	118	111	95	229	179	139	347	290	234
	10	101	89	101	244	173	167	345	262	268
56.9	0	123	123	123	249	249	249	372	372	372
	5	90	118	111	296	258	249	386	376	360
	10	90	68	96	383	317	295	473	385	391
46.6	0	113	113	113	329	329	329	442	442	442
	5	80	71	132	367	370	373	447	441	505
	10	87	96	121	329	330	349	416	426	470
37.8	0	95	95	95	416	416	416	511	511	511
	5	53	120	117	460	432	394	513	552	511
	10	67	107	127	466	472	386	533	579	513
24.8	0	55	55	55	403	403	403	458	458	458

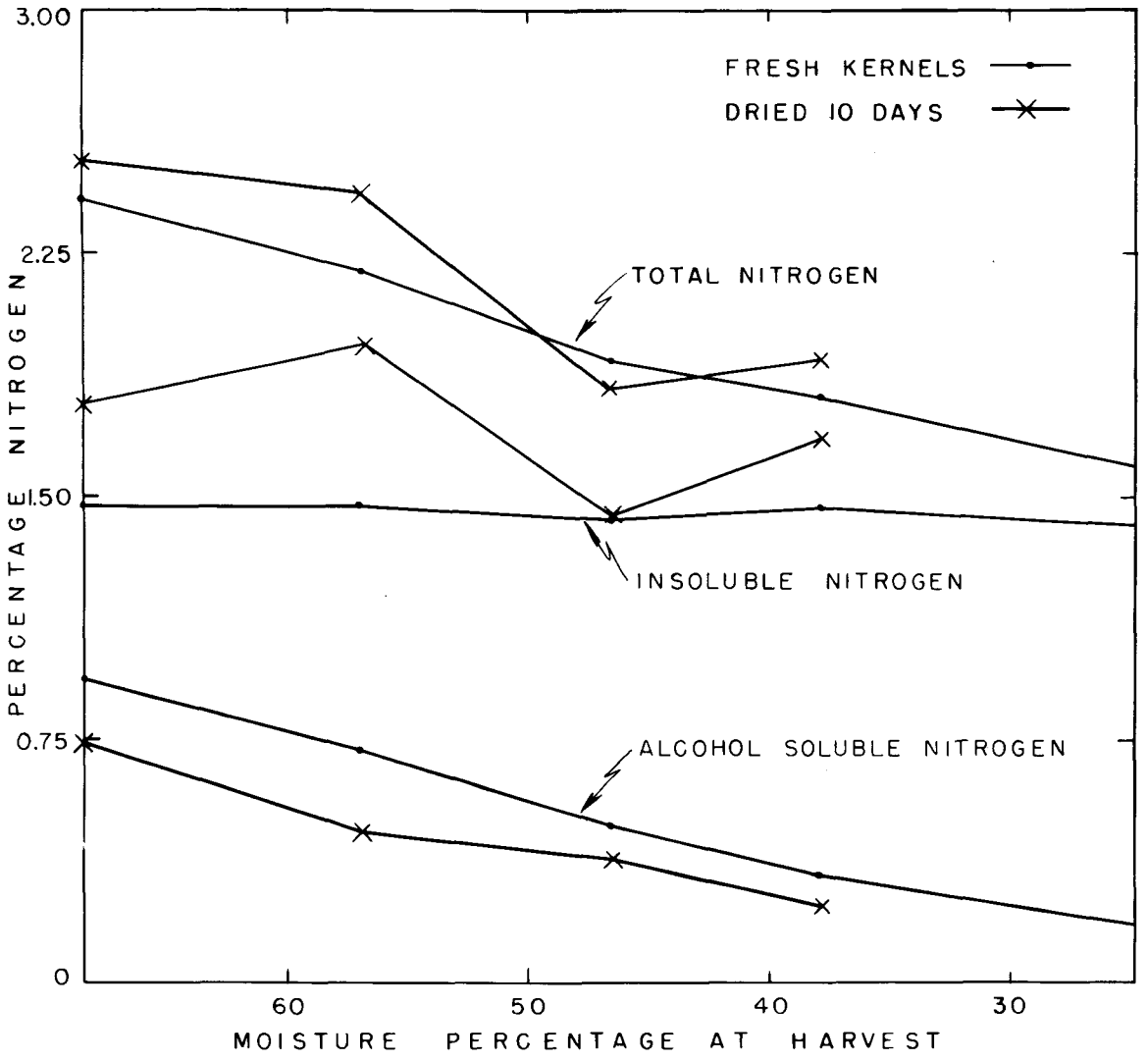

Fig. 5 Effect of maturity and drying on the percentages of nitrogen fractions in the dry matter of kernels, 1948

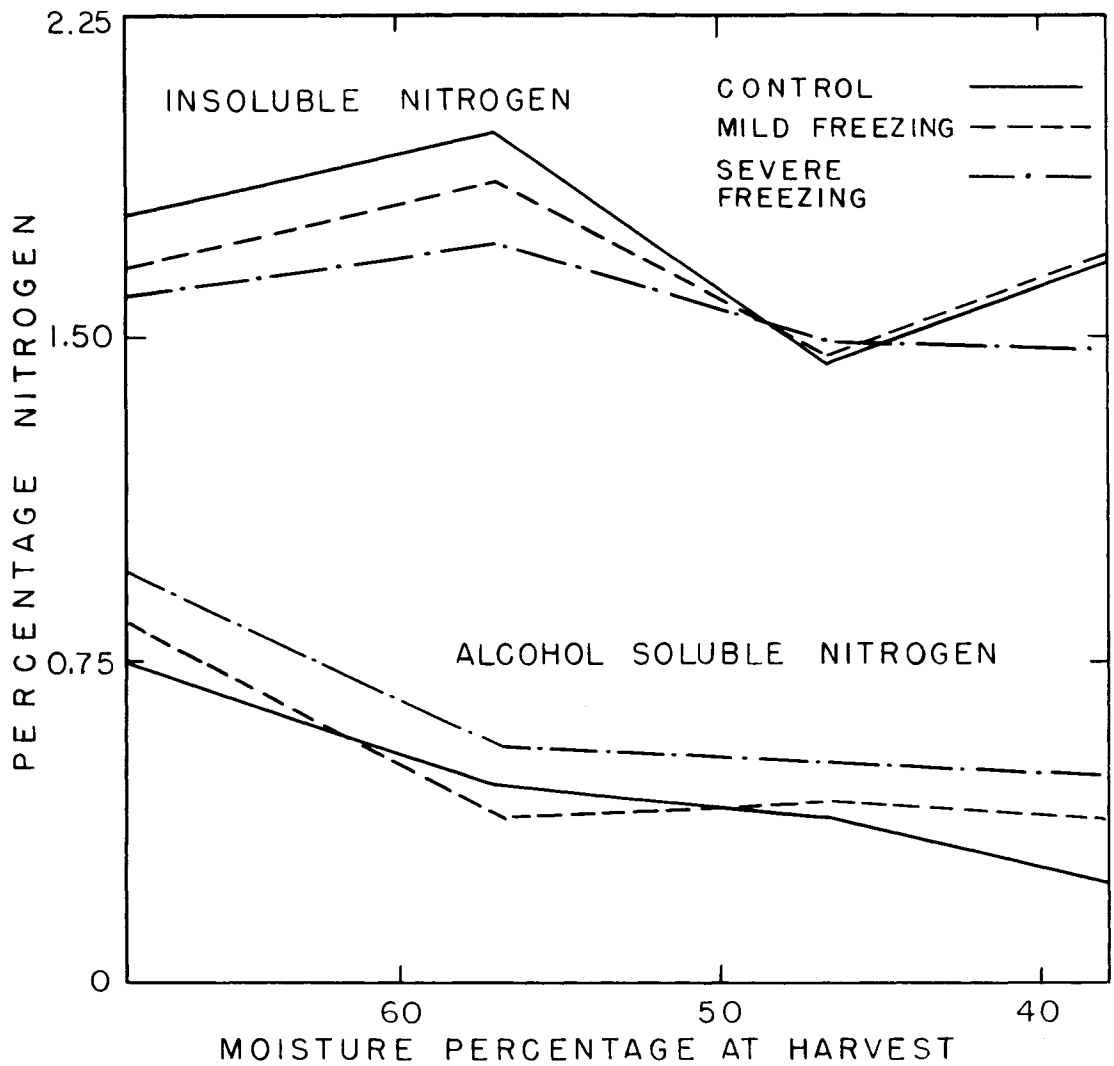

Fig. 6 Effect of freezing on the percentage of nitrogen fractions in the dry weight of kernels after 10 days drying in the field, 1948.

observed that quantities of total and insoluble nitrogen increased to a maximum at 57 per cent moisture, then gradually decreased. The field drying resulted in maintenance or decrease of the alcohol-soluble nitrogen, and in increase of the insoluble and total nitrogen.

DISCUSSION

Robinson (55) found that the maximum dry weight in corn was attained when the grain contained approximately 40 per cent moisture. Rossman (56), using the absolute dry weight, showed that dry weight for all varieties he used increased after 40 per cent moisture. The increase differed with the different varieties and was more affected by the maternal than the paternal parent in the crosses.

Shaw (62) criticized the method of absolute dry weight for its inconsistency. He used the ratio

$$R = \frac{\text{Dry weight of grain}}{\text{Total dry weight of ear}}$$

assuming that this ratio would be constant at complete maturity or maximum dry weight. He found that maximum dry weight in grain was obtained at 29.2, 35.2, and 40.0 per cent moisture for early, medium, and late varieties, respectively. Between years these values varied no more than 1.5 per cent.

In previous work by the writer (1), the dry weight for 100 kernels was found to increase from 24.8 grams at 41.0 per cent moisture, to 28.3 grams at 35.4 per cent moisture, and to 32.3 grams at 24.9 per cent moisture in early plants. In the late plants for the same variety, the weight was 22.2 grams at 40 per cent moisture and remained the same at 26.8 per cent moisture.

Tables 2 and 3 show that in 1947 the dry weight of 100 kernels increased from 25.0 grams at 39.5 per cent moisture to 28.5 grams at 31.8 per cent moisture. For the same variety in 1948 the weight was 28.7 grams at 37.8 per cent moisture and remained the same at 24.8 per cent moisture. The maximum dry weight is not attained when moisture reaches 35 per cent (3) or 40 per cent (54, 55), but it is a matter of varietal and seasonal differences.

The faster rate of drying in frozen ears, 1950, when husks were removed, is what might be expected. With freezing, ice crystals are formed in the intercellular spaces and water is drawn from the inside. Cell permeability is increased by freezing (24, 57). After thawing, an accumulation of water outside the cells is readily available for evaporation. In late stages of maturity the cobs are higher in moisture content than the kernels (table 1) and (63). The first ice crystals to form are probably located in the cobs, to which water from the kernel may be drawn. The cob being a spongy tissue with large intercellular spaces dries rapidly and so the frozen kernels lose their moisture faster than the unfrozen.

When the husks are left on during drying, the situation is different. The husks are killed by freezing and dry quickly. They tend to stick to the ear and do not expose it to the atmosphere as do the unfrozen. The dried

dead tissue tightly enveloping the ear decreases evaporation. Hardened tissues are believed to have a high water retaining power due to the accumulation of hydrophilic colloids (47). It was shown here that frozen corn contained more reducing sugars and non-protein nitrogenous compounds than the unfrozen. The faster drying rate in the severely frozen ears compared to the mildly frozen ones may be due to increased permeability.

The dry weight of 100 kernels did not change with drying or freezing in late harvests. Apparently there is a balance between the decrease of dry weight by respiration and the increase by translocation from cobs and husks. In early harvests, however, there was a slight decrease in the dry weight of the frozen samples. This result agrees with those of Newton and McCalla (50), Waldron (70), and Livington and Swinbank (39) on wheat. Newton and McCalla explained that in the early stages the frosted samples lost more by stoppage of translocation than they saved by stoppage of respiration. Bailey and Gurjar (5) found that rate of respiration was greater in frosted wheat kernels than in sound ones. Here the frozen kernels lost weight by both stoppage of translocation and increased respiration.

It is known from previous work (14, 33, 55) that corn kernels contain more sucrose than reducing sugars throughout development, and that both sugars decrease with maturity.

These results are confirmed here. Loomis (40) showed that sucrose is the translocation form most abundant in m ize. He also demonstrated that sucrose moved from leaves to sheaths, to stalks, to ear shanks, and to the kernels, against concentration gradients. It could be added also that sugars in harvested ears decreased greatly on drying. The rate of decrease in reducing sugars was fairly slow, as was indicated by the significant difference in the percentage between 5 and 10 days of drying (table 11). Sucrose, however, decreased rapidly at the beginning, then more slowly. Some causes for sugar depletions are; (a) hydrolysis of sucrose, (b) break-down of monosaccharides in respiration, and (c) polymerization of reducing sugars into polysaccharides. There are also factors by which sugars could be increased, i. e. translocation and hydrolysis of di- or polysaccharides.

Blish (10) and Newton and McCalla (50) found that after drying, frozen wheat contained more reducing sugars than unfrozen. Sucrose was not affected by freezing. Data presented here for 1947, 1948, and 1950 and previous work (1) show that the reducing sugars were higher in the frozen corn. With regard to sucrose, the frozen samples of 1947 and 1948 contained less than the unfrozen. The 1950 samples, however, showed no difference in sucrose content due to freezing. It should be remembered that the 1950 samples

were dried quickly in the laboratory with no husks, while both the 1947 and 1948 were dried slowly in the field without husking.

The changes in sugars of frozen corn kernels after drying indicated that enzymes responsible for these changes were still functioning. Enzymes of various types are known to withstand low temperatures (15, 28, 52, 53).

Bailey and Gurjar (5) suggested that the disorganization of the protoplasm of wheat by frost resulted in the reduction of its synthetic activity. They claimed that hydrolytic enzymes were activated by frost and this activation led to an accumulation of split products. McCalla and Newton (48) accepted this hypothesis on the basis of their results in wheat.

In the hardening process, plants usually accumulate split products (47, 57). It was noted here, however, that sucrose was less in the frozen kernels. This rapid loss of sucrose might have been a result of an increased hydrolysis that took place in frozen kernels. Translocation of sucrose from cobs and husks to kernels was, at the same time, decreased by freezing.

With regard to reducing sugars the picture is more complicated. It could be visualized, however, that some

of the reducing sugars lost in respiration are compensated for by translocation or by hydrolysis of sucrose and other compounds. Compensation is suggested by; (a) the slow rate of decrease in reducing sugars, and (b) the fact that the percentage of reducing sugars was sometimes higher in dried ears than in fresh, (table 9, columns 3, 4, and 5). Sources of reducing sugars in kernels of snapped ears are; (a) translocation from cobs and husks, (b) hydrolysis of sucrose, and (c) hydrolysis of polysaccharides. One or more of the three may be involved. If hydrolysis of sucrose is the most important factor, the assumption that this hydrolysis is increased by freezing would explain why frozen kernels contain relatively more reducing sugars.

The total nitrogen percentage in kernels was shown here to decrease gradually with maturation. This result agrees with those of Evans (20) and Zeleny (74). The latter's work indicated that the percentage of alcohol-soluble nitrogen with respect to total nitrogen increased with maturity. Data for 1947, when the same alcohol concentration as that of Zeleny was used (80 per cent), gave a percentage ratio of 34 when moisture content of kernels was 65 per cent. The percentage increased to 43 at 58 per cent moisture, remained constant until 40 per cent moisture was reached, and then dropped to 31 at 32 per cent moisture. In the 1948 data, however, when 70 per cent alcohol was

used for extraction, the percentage ratio decreased gradually from 39.0, to 33.0, to 25.5, to 18.5, to 11.8, at moisture contents of 69, 57. 47, 38, and 25 per cent, respectively.

Some of the causes of such contradictory results are; (a) difference in alcohol concentration, (b) varietal and seasonal differences, and (c) the inclusion in the alcohol-soluble fraction of the non-protein nitrogen which was separated in Zeleny's material before extraction with alcohol. Because the samples in this work were stored in alcohol, some difficulties were encountered in separating the non-protein fraction. In some samples, however, t he separation of the non-protein nitrogen was attempted by precipitating the protein with acetic acid after driving off the alcohol. Data were not consistent, but there was an indication that the non-protein nitrogen percentage decreased with maturity and during drying after harvest; the decrease uring drying was slowed by freezing. It is believed that the results here support Zeleny's observations that water-soluble, non-protein nitrogen decreased with maturity, while zein increased, and the insoluble globulin and glutelin remained constant.

When ears were dried in the field the alcohol- (70 per cent) soluble nitrogen percentage decreased, though not significantly, while the insoluble and the total nitrogen percentages increased. Reasons for increase of total

nitrogen by drying could be either loss of total dry matter per kernel, or translocation of nitrogen from cobs and husks to the kernels, or both.

Discussion of nitrogen is based mostly on 1948 data because the sampling was more uniform and results more consistent. The data of table 17 indicate that the decrease in alcohol-soluble percentages by drying was not significant, but the comparison for drying effect was between the fresh controls and the dried controls and frozen together. The decrease in the alcohol-soluble nitrogen of the controls was balanced by no decrease or a slight increase in that of the frozen. The mean of the alcohol-soluble nitrogen of the fresh samples in the first 4 harvests (table 16, column 3) = 0.617, that of the 5 days drying for the same harvests (same column) = 0.491 and that of the 10 days drying = 0.452. The standard error is calculated to be

$$\sqrt{\frac{2 \times 0.006776}{8}} = 0.04116.$$

The t values for difference between means = $\frac{0.617-0.491}{0.04116} = 3.061$ and $\frac{0.617-0.452}{0.04116} = 4.009$ with 24 d. f., both are highly significant for the effect of 5 or 10 days drying. We can assume, then, that there is an actual decrease of alcohol-soluble nitrogen during drying of unfrozen ears.

As mentioned before (tables 16, 17, 18, and 19), the frozen ears contained more alcohol-soluble, and less

insoluble nitrogen after drying than the unfrozen, the total nitrogen being the same in both. These results are confirmed by the 1950 data (table 20). Studies on wheat indicated that slight frost did not cause any change in total or fractions of nitrogen (48, 61). The wheat frozen severely at early stages, however, contained a lower percentage of total nitrogen (48) and more non-protein and salt-soluble nitrogen than the unfrozen (10, 48, 59, 60).

McCalla and Newton (48) believed that respiration was reduced in the frost-injured germ during subsequent drying, and thus a higher C/N ratio occurred in dried kernels. The same injury checked synthesis and resulted in larger fractions of non-protein and salt-soluble nitrogens. Sharp (59) goes further and explains that the synthetic reaction of complex proteins from amino compounds is normally irreversible, and that this reaction is rendered reversible by freezing with a tendency to form more amino compounds.

The picture of nitrogen distribution in frozen corn kernels could be drawn as follows. When the harvested ears are left to dry, nitrogen in simple forms is translocated into the kernels, where it is directly synthesized into more complex forms such as zein, and particularly glutelins and globulins. If ears are frozen, the translocation does not cease but the rate of synthesis is decreased if not completely stopped.

The decrease in germinability of frozen seed corn has been shown to depend on many factors (32, 56). Data in table 8 show that the fresh weight of seedling epicotyls increased with maturity and, within one harvest, decreased with the degree of freezing injury. Rossman (56) obtained the same results, although when he soaked mature kernels before freezing he noticed that the injury by freezing was an all or none effect. In other words the kernels that survived freezing did not show any decrease in seedling weights. He stated:

> Quicker killing, no reduction in seedling vigor, and no increase in percentage of weak seedlings when soaked seed was frozen suggest that death may have resulted from rapid intracellular ice formation. In contrast to these results, shelled seed in the fresh condition was more slowly killed by freezing, suggesting a closer association of water with the protoplasm and more resistance to crystallization.-----
>
> Several effects were noted: reduction in seedling vigor, abnormal seedlings, and finally complete death of the seed. The progressive type of injury occurred, accompanied by dehydration of the protoplasm and eventually an irreversible physico-chemical change of the protoplasm. Localized killing, as evidenced by the failure of some frozen seed to develop either a plumule, a radicle, or more than one or two leaves suggests that localized intracellular ice formation may have occurred in the embryo. Reduction in seedling vigor may not carry over into the yield of grain grown from frozen seed.----

Other mature seeds, however, could have a progressive type of injury. Busse and Burnham (11) found that among the dormant seeds of different species treated with liquid air, only flax and cotton exhibited abnormalities in the

growing seedlings. Since cotton and flax seed are rich in oil and glycerides, their protoplasm is more hydrophylic.

It seems that reduced vigor may be due either to quantitative or qualitative properties of the protoplasm and nutrients. That is, the reduction in vigor of immature seed is mostly due to less protoplasm in the embryo and reserve; the reduction in vigor of frozen seed due to the change of protoplasmic properties of the embryo.

SUMMARY AND CONCLUSIONS

1. The physiological changes in maturing corn were studied.
2. The physical and chemical changes in dried kernels from ears harvested at different stages of development were compared with those occurring in frozen kernels.
3. Maximum dry weight of kernels was attained at different moisture levels depending on variety and season.
4. Under field conditions, ears snapped and frozen dried more slowly than the unfrozen.
5. Both reducing sugars and sucrose decreased in the kernels with maturation.
6. In the drying ears, sucrose in kernels decreased rapidly but reducing sugars decreased slowly. When ears were frozen before drying, kernels contained less sucrose but more reducing sugars.
7. During maturation, the alcohol-soluble nitrogen and the total nitrogen percentages decreased while the insoluble nitrogen remained constant.
8. With field drying, the alcohol-soluble nitrogen decreased while both the insoluble and total nitrogen increased. The frozen ears, however, contained more alcohol-soluble, less insoluble, and the same percentage of total nitrogen as the unfrozen.
9. Freezing slowed the synthesis of protein but did not

stop nitrogen translocation to the kernels.

10. Ability of corn kernels to germinate starts early in their life, but the more mature seed produced more vigorous seedlings.

11. Freezing decreased both germinability and viability of seed corn. Reduction of vigor may be due either to protoplasmic injury or to a reduction in seed reserves.

LITERATURE CITED

1. Aboul-Ela, M. M. Physiological changes in maturing maize. Unpublished M. S. Thesis. Library, Iowa State College, Ames, Iowa. 1947.

2. Adams, J. The effect of very low temperatures on moist seeds. Sci. Proc. Roy. Dub. Soc. New Ser. 11:1-6. 1905.

3. Aldrich, S. R. Maturity measurements in corn and an indication that grain development continues after premature cutting. Jour. Amer. Soc. Agron. 35: 667-680. 1943.

4. Andrew, R. H., R. A. Brink, and N. P. Neal. Some effects of the waxy and sugary genes on the endosperm development in maize. Jour. Agr. Res. 69:355-372. 1944.

5. Bailey, C. H. and A. M. Gurjar. Respiration of stored wheat. Jour. Agr. Res. 18:685-713. 1918.

6. Barnes, W. H. and F. W. Mathews. A note on the diffraction of x-rays by vitrified and by frozen gelation gels. Biodynamica Vol. 2 No. 49. 1939.

7. Becquerel, P. Rôle de la synérèse dans le mécanisme de la congélation cellulaire. Chronica Botanica 5:10-11. 1939.

8. Bennett, Norah and W. E. Loomis. Tetrazolium chloride as a test reagent for freezing injury of seed corn. Plant. Physiol. 24:162-174. 1949.

9. Bernstein, L. Amyloses and carbohydrates in developing maize endosperm. Amer. Jour. Bot. 30:517-526. (date ??)

10. Blish, M. J. Effect of premature freezing on composition of wheat. Jour. Agr. Res. 19:181-188. 1920.

11. Busse, W. G. and C. R. Burnham. Some effects of low temperatures on seeds. Bot. Gaz. 90:399-411. 1930.

12. Chambers, R. and H. P. Hale. The formation of ice in protoplasm. Proc. Roy. Soc. Lond. Ser. B 110:337-352. 1932.

13. Csonka, F. A. Amino acids in the corn kernel. Jour. Agr. Res. 59:765-768. 1939.

14. Culpepper, C. W. and C. A. Magoon. Studies upon the relative merits of sweet corn varieties for canning purposes and the relation of maturity of corn to the quality of the canned product. Jour. Agr. Res. 28:403-443. 1924.

15. d'Arsonval, M. Action Physiologique des très basses températures. Compt. Rend. Soc. Biol. 808-809. 1892.

16. De Candolle, C. Sur la vie latente des graines. Arch. Sci. Phys. Nat. Ser. 3, 33:497-512. 1895.

17. Dessureaux, L., N. P. Neal, and R. A. Brink. Maturation in corn. Jour. Amer. Soc. Agron. 40:733-745. 1948.

18. Earl, F. R., J. J. Curtis, and J. E. Hubbard. Composition of the component parts of the corn kernel. Cereal Chem. 23:504-511. 1946.

19. Eckerson, S. H. Microchemical studies in the progressive development of the wheat plant. Wash. Agr. Exp. Sta. Bul. 139. 1917.

20. Evans, W. James. Changes in the biochemical composition of the corn kernel during development. Cereal Chem. 18:468-473. 1941.

21. Goetz, A. and S. S. Goetz. Death by devitrification in yeast cells. Biodynamica Vol. 2, No. 43. 1938.

22. Hansen, D. W., B. Brimhall, and G. F. Sprague. Relationship of zein to the total protein in corn. Cereal Chem. 23:329-334. 1946.

23. Harlan, H. V. and M. N. Pope. The germination of barley seed harvested at different stages of growth. Jour. Hered. 13:72-75. 1922.

24. Harvey, R. B. Hardening process in plants and development from frost injury. Jour. Agr. Res. 15:83-111. 1918.

25. Hassid, W. Z. Determination of reducing sugars and sucrose in plant material. Ind. Eng. Chem. Anal. Ed. 8:138-140. 1936.

26. _____ Determination of sugars in plants by oxidation with ferricyanide and ceric sulfate titration. Ind. Eng. Chem. Anal. Ed. 9:228-229. 1937.

27. Helgeson, E. A. and K. L. Blanchard. The relation of moisture content to freezing injury in Rival and Mindum wheats. N. D. Agr. Exp. Sta. Bimon. Bul. 4:15-16. 1942.

28. Hepburn, J. S. V. The occurrence of catalase, oxidases, and reductases in the fat of the common fowl (Gallus Domesticus). U. S. D. A. Bur. Chem. Cir. 103:6-12. 1912.

29. Hopkins, C. G., L. H. Smith, and E. M. East. The structure of the corn kernel and the composition of its different parts. Ill. Agr. Exp. Sta. Bul. 87. 1903

30. Hopper, T. H. Composition and maturity in corn. N. D. Agr. Exp. Sta. Bul. 192. 1925.

31. Jones, W. J. and H. A. Huston. Composition of maize at various stages of its growth. Ind. Agr. Exp. Sta. Bul. 175. 1914.

32. Kiesselbach, T. A. and J. A. Ratcliff. Freezing injury of seed corn. Nebr. Agr. Exp. Sta. Res. Bul. 16. 1920.

33. Lampe, Lois. A microchemical and morphological study of the developing endosperm of maize. Bot. Gaz. 91:337-376. 1931.

34. Larmour, R. K. A comparative study of the glutelins of cereal grains. Jour. Agr. Res. 35:1091-1120. 1927.

35. Levitt, J. Frost killing and hardiness of plants. Minneapolis, Burgess Publishing Co. 1941.

36. Lind, E. F. Clearing and deleading of plant extracts for reducing sugar determination. Unpublished Ph. D. Thesis. Library, Iowa State College, Ames, Iowa. 1949.

37. Lipman, C. B. Normal viability of seeds and bacterial spores after exposure to temperatures near the absolute zero. Plant Physiol. 11:201-205. 1936.

38. _____ On the difference in resistance of various types of cells to extremely low temperatures. Biodynamica Vol. 2, No. 45. 1939.

39. Livington, J. E. and J. C. Swinbank. Some factors influencing the injury to winter wheat heads by low temperatures. Agron. Jour. 42:153-157. 1950.

40. Loomis, W. E. Translocation of carbohydrates in maize. Sci. 101:398-400. 1945.

41. Luyet, B. J. and H. M. Condon. Temperature relationships and ice-water proportions during death by freezing plant tissues. Biodynamica Vol. 2, No. 37. 1938.

42. Luyet, B. J. and P. M. Gehenio. The lower limit of vital temperatures. A review. Biodynamica Vol. 1, No. 33. 1938.

43. _____ and _____. The survival of moss vitrified in liquid air and its relation to water content. Biodynamica Vol. 2, No. 42. 1938.

44. _____ and _____. The physical state of protoplasm at low temperatures. Review and critical study. Biodynamica Vol. 2, No. 48. 1939.

45. _____ and _____. The mechanism of injury and death by low temperature. A review. Biodynamica Vol. 3, No. 60. 1940.

46. Luyet, B. J. and M. C. Gibbs. On the mechanism of congelation and of death in the rapid freezing of epidermal cells. Biodynamica Vol. 1, No. 25. 1937.

47. Maximov, N. A. Internal factors of frost and drought resistance in plants. Protoplasma 7:259-291. 1929.

48. McCalla, A. G. and R. Newton. Effect of frost on wheat at progressive stages of maturity. II Composition and biochemical properties of grain and foour. Can. Jour. Res. Vol. 13, Sec. C:1-31. 1935.

49. Miller, E. C. A physiological study of the winter wheat plant at different stages of its development. Kans. Agr. Exp. Sta. Tech. Bul. 47. 1939.

50. Newton, R. and A. G. McCalla. Effect of frost on wheat at progressive stages of maturity. I Physical characteristics of the kernels. Can. Jour. Res. 10:414-429. 1934.

51. Osborne, T. B. and S. H. Clapp. Hydrolysis of the proteins of maize, Zea mays. Amer. Jour. Physiol. 20:477-493. 1907.

52. Pennington, M. E. and J. S. Hepburn. Studies on chicken fat. Jour. Amer. Chem. Soc. 34:212-222. 1912.

53. Pozerski, M. Action de quelques ferments solubles après reffroidissement vers -191 degrés au moyen de l'air liquide. Compt. Rend. Soc. Biol. 714-716. 1900.

54. Rather, H. C. and A. R. Marston. A study of corn maturity. Mich. Agr. Exp. Sta. Quar. Bul. 22:278-288. 1940.

55. Robinson, F. L. Physiologic factors affecting the germination of seed corn. Ia. Agr. Exp. Sta. Tech. Bul. 176. 1934.

56. Rossman, E. C. Freezing injury of maize seed. Plant Physiol. 24:629-656. 1949.

57. Scarth, G. W. Cell physiological studies of frost resistance: A review. New Phyt. 43:1-12. 1944.

58. Schaible, P. J. Composition of certain hybrid and open-pollinated corns and their performance in poultry rations. Mich. Agr. Exp. Sta. Quar. Bul. 29:31-29. 1946.

59. Sharp, P. F. Wheat and flour studies, III. The amino nitrogen content of the immature wheat kernel and the effect of freezing. Cereal Chem. 2:12-38. 1925.

60. _____ The composition of wheat and mill products from frozen and non-frozen wheat harvested at various stages of maturity. Cereal Chem. 3:402-410. 1926.

61. _____ and R. Elmer. Wheat and flour studies. I. Proteolytic enzymes of flour I. Auto-digestion of flour milled from frozen and non-frozen wheat harvested at various stages of maturity. Cereal Chem. 1:83-106. 1924.

62. Shaw, R. H. Studies on corn phenology and maturity in Iowa. Unpublished Ph. D. Thesis. Library, Iowa State College, Ames, Iowa. 1949.

63. _____ and W. E. Loomis. Bases for the prediction of corn yields. Plant. Physiol. 25:225-244. 1949.

64. Shelton, E. M. Harvesting for fodder and corn. Kans. Agr. Exp. Sta. Ann. Report 2:22-29. 1889.

65. Sherwood, L. V. A physiological study of cold tolerance in corn. Jour. Amer. Soc. Agron. 29:1022-1030. 1937.

66. Showalter, M. F. and R. H. Carr. Characteristic proteins in high- and low-protein corn. Jour. Amer. Chem. Soc. 44:2019-2023. 1922.

67. Spitzer, G., R. H. Carr, and W. F. Epple. Soft corn-its chemical composition and nitrogen distribution. Jour. Amer. Chem. Soc. 41:1212-1221. 1919.

68. Thatcher, R. W. The progressive development of the wheat kernel. Jour. Amer. Soc. Agron. 5:203-213. 1913.

69. _____ The progressive development of the wheat kernel II. Jour. Amer. Soc. Agron. 7:273-283. 1915.

70. Waldron, L. R. Differences of injury by frost to wheat plants grown comparably. Jour. Amer. Soc. Agron. 24:494-500. 1932.

71. Whitcomb, W. O. and P. F. Sharp. Germination of frozen and non-frozen wheat harvested at various stages of maturity. Jour. Agr. Res. 31:1179-1188. 1925.

72. Wolf, M. J., M. M. MacMasters, J. E. Hubbard, and C. E. Rist. Comparison of corn starches at various stages of kernel maturity. Cereal Chem. ~~15~~ 25:312-325. 1948.

73. Woodman, H. E. and F. L. Engledow. A chemical study of the development of the wheat grain. Jour. Agr. Sci. 14:563-586. 1924.

74. Zeleny, L. The distribution of nitrogen in the seed of *Zea mays* at different stages of maturity. Cereal Chem. 12:536-542. 1935.

ACKNOWLEDGMENT

The writer is indebted to Dr. W. E. Loomis for suggesting the problem and helping in preparation of the manuscript. He also wishes to thank Miss Norah Bennett for the germination tests she made, and Mr. P. Homeyer for his advice in statistical analysis.